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(54) Title: INHIBITORS OF CYCLOPHILIN ROTAMASE ACTIVITY (57) Abstract This invention relates to the method of using neurotrophic cyclophilin inhibitor compounds having an affinity for cyclophilin-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.		

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Title:

INHIBITORS OF CYCLOPHILIN ROTAMASE ACTIVITY

1. Field of the Invention

5 This invention relates to the method of using
neurotrophic cyclophilin inhibitor compounds having an
affinity for cyclophilin immunophilins as inhibitors
of the enzyme activity associated with immunophilin
proteins, and particularly inhibitors of peptidyl-
10 prolyl isomerase or rotamase enzyme activity of the
cyclophilins.

2. Description of the Prior Art

15 The term immunophilin refers to a number of
proteins that serve as receptors for the principal
immunosuppressant drugs, cyclosporin A (CsA), FK506,
and rapamycin. Known classes of immunophilins are
cyclophilins, and FK506 binding proteins, such as
FKBP. Cyclosporin A binds to cyclophilin while FK506
20 and rapamycin bind to FKBP. These immunophilin-drug
complexes interface with a variety of intracellular
signal transduction systems, especially in the immune
system and the nervous system.

25 Immunophilins are known to have peptidyl-prolyl
isomerase (PPIase) or rotamase enzyme activity. It
has been determined that rotamase activity has a role
in the catalyzation of the interconversion of the cis
and trans isomer of immunophilin proteins.

30 Immunophilins were originally discovered and
studied in immune tissue. It was initially postulated

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by those skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Instead immunosuppression appears to stem from the formulation of a complex of immunosuppressant drugs and immunophilins. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibiting T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system. Within neural tissues, immunophilins appear to influence nitric oxide synthesis, neurotransmitter release, and neuronal process extension.

Surprisingly, it has been found that picomolar concentrations of an immunosuppressant such as

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cyclosporin A stimulates neurite out growth in PC12 cells and sensory neurons, namely dorsal root ganglion cells (DRGs). More particularly, it has been found that drugs with a high affinity for cyclophilin are
5 potent rotamase inhibitors and exhibit excellent neurotrophic effects. Snyder et al., "Immunophilins and the Nervous System", *Nature Medicine*, Volume 1, No. 1, January 1995, 32-37. These findings suggest the use of inhibitors of cyclophilin rotamase activity
10 in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease may occur due to the
15 loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system
20 have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat SDAT patients with exogenous nerve growth factor or other neurotrophic
25 proteins such as brain derived growth factor, glial derived growth factor, ciliary neurotrophic factor, and neurotrophin-3 to increase the survival of degenerating neuronal populations.

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Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, *J. Am. Soc. Nephrol.* 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, *N. Engl. J. Med.* 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 *N. Engl. J. Med.* 321: 1725).

The present invention provides both immunosuppressive and non-immunosuppressive cyclophilin inhibitor compounds containing small molecule cyclophilin rotamase inhibitors which are extremely potent in augmenting neurite outgrowth, and for promoting and stimulating neuronal growth and regeneration in various neuropathological situations where neuronal repair can be facilitated. These situations include augmenting neurite outgrowth and promoting neuronal growth and regeneration for nerve

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damage by physical injury or disease state such as diabetes, including peripheral nerve damage, damage to motor neurons, damage to the central nervous system (spinal cord and brain) including damage to spinal
5 neurons and neurons in the brain, brain damage associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease and Alzheimer's disease.

10

SUMMARY OF THE INVENTION

This invention relates to the method of using neurotrophic cyclophilin inhibitor compounds having an affinity for cyclophilin-type immunophilins as
15 inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

A preferred embodiment of this invention is a method of treating a neurological activity in an
20 animal, comprising:

administering to an animal an effective amount of an immunosuppressive cyclosporin represented by formula I and pharmaceutically acceptable salts thereof, wherein:

25 R^1 is MeBmt or [3-DesoxyMeBmt] or represented by formula III; wherein positions 2, 3, and 4 have the configurations S, R and R, respectively;

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wherein -X-Y- is connected by a double bond
or a single bond; wherein:

5 R^2 is independently selected from the group
consisting of Abu, Ala, Thr, Val, and norVal; R^3
is Sar; R^4 is MeLeu; R^5 is Val; R^6 is MeLeu; R^7 is
Ala; R^8 is D-Ala; R^9 is MeLeu; R^{10} is MeLeu; and,
 R^{11} is Val or [D-MeVal].

10 Another preferred embodiment of this invention is
a method of treating a neurological activity in an
animal, comprising:

administering to an animal an effective amount of
a non-immunosuppressive cyclosporin of formula IV:
wherein:

15 R^1 is MeBmt or dihydro MeBmt or represented by
formula III; wherein positions 2, 3, and 4 have
the configurations S, R and R, respectively;
wherein -X-Y- is connected by a double bond or a
single bond; wherein: R^2 is Abu or a fluorinated
analog thereof; R^3 is Sar, D-MeAla, or a
20 fluorinated analog thereof; R^4 is an N-methylated
amino acid residue with a (C1-C9) straight or
branched chain alkyl or alkenyl group; these
straight or branched alkyl or alkenyl groups may
be substituted by cycloalkyl (C3-C8); R_1 may also
25 be (C3-C8) cycloalkyl or (C5-C7) cycloalkenyl;
the above alkyl, alkenyl, cycloalkyl or
cycloalkenyl groups may be substituted with (C1-
C4) alkyl or (C1-C4) alkenyl, or hydroxy,

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especially where R⁴ is MeLeu, MeVal, Me homo-Ala, or [Me-(α -methyl)Thr]; R⁵ is Val or a fluorinated analog thereof; R⁶ is MeAla, MeAbu, or a fluorinated analog thereof; R⁷ is Ala or a fluorinated derivative thereof; R⁸ is (a) D-Ala or a fluorinated analog thereof; or (b) O-acyl-D-Ser or O-acyl-D-Thr wherein the acyl group is defined as R¹² -CO- where R¹² represents hydrogen, C₁₋₆alkyl, phenyl or substituted phenyl of formula V wherein X¹ and X² independently are (a) C₁₋₆alkyl; (b) C₁₋₆alkanoyl; (c) CH₂OH; (d) halo; (e) C₁₋₆alkoxy; (f) -NH₂; (g) -NO₂; (h) -COOH; (i) -COOC₁₋₆alkyl; or (j) -H; R⁹ and R¹⁰ are independently MeLeu or a fluorinated analog thereof; and, R¹¹ is MeVal or a fluorinated analog thereof.

Another preferred embodiment of this invention is a method of treating a neurological disorder in an animal, comprising:

administering to an animal an effective amount of a cyclophilin inhibitor having an affinity for cyclophilin-type immunophilins to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the cyclophilin-type immunophilin exhibits rotamase activity and the cyclosporin derivative inhibits said rotamase activity of the immunophilin.

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Another preferred embodiment of this invention is a method of treating a neurological disorder in an animal, comprising:

5 administering to an animal an effective amount of a cyclophilin inhibitor having an affinity for cyclophilin-type immunophilins in combination with an effective amount of a neurotrophic factor selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, ciliary neurotrophic factor, and neurotrophin-3, to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the cyclophilin-type immunophilin exhibits rotamase activity and the cyclosporin derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of stimulating growth of damaged peripheral nerves, comprising;

20 administering to damaged peripheral nerves an effective amount of a cyclophilin inhibitor compound having an affinity for cyclophilin-type immunophilins to stimulate or promote growth of the damaged peripheral nerves, wherein the cyclophilin-type immunophilins exhibit rotamase activity and the cyclosporin derivative inhibits said rotamase activity of the immunophilin.

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Another preferred embodiment of this invention is a method of stimulating growth of damaged peripheral nerves, comprising:

administering to damaged peripheral nerves an
5 effective amount of an FKBP inhibitor compound having
an affinity for cyclophilin-type immunophilins to
stimulate growth of damaged peripheral nerves, wherein
the cyclophilin-type immunophilin exhibit rotamase
activity and the cyclosporin derivative inhibits said
10 rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method for promoting neuronal regeneration and growth in animals, comprising:

administering to an animal an effective amount of a
15 cyclophilin inhibitor compound having an affinity for
cyclophilin-type immunophilins to promote neuronal
regeneration, wherein the cyclophilin-type
immunophilins exhibit rotamase activity and the
cyclosporin derivative inhibits said rotamase activity
20 of the immunophilin.

Yet another preferred embodiment of this invention is a method for preventing neurodegeneration in an animal, comprising:

administering to an animal an effective amount of a
25 cyclophilin inhibitor having an affinity for
cyclophilin-type immunophilins to prevent
neurodegeneration, wherein the cyclophilin-type
immunophilin exhibits rotamase activity and the

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cyclosporin derivative inhibits said rotamase activity of the immunophilin.

BRIEF DESCRIPTION OF THE DRAWINGS

5 FIG. 1 is dose-response curve for Cyclosporin A. FIG. 1 shows a typical dose at which ED_{50} for CsA, the dose at which 50% of the maximal response was elicited, is calculated to be 50 nM.

DETAILED DESCRIPTION OF THE INVENTION

10 The novel neurotrophic cyclophilin inhibitor compounds of this invention have an affinity for the cyclosporin binding protein, cyclophilin. When the neurotrophic compounds of the invention are bound to
15 cyclophilin, they have been found to inhibit the prolyl-peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and unexpectedly stimulate neurite growth.

 The compounds of the present invention can be
20 used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate,
25 cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-

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hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalensulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans

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for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, the stimulation of growth of damaged peripheral nerves, the stimulation of growth of damaged neurons in the spinal cord, the stimulation of growth of motor nerves, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed intervertebral disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, porphyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally,

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buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes
5 subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effectively administered by an
15 intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated
20 according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for
25 example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils

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are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug.

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Such materials include cocoa butter, beeswax and polyethylene glycols.

5 The compounds of this invention may also be administered optically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

10 For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the
15 compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment
20 containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively,
25 the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate,

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polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about 0.1mg to about 10,000mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1mg to about 1000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotrophin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated

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and the neurotrophic effectiveness of the drug combination.

Methods and Procedures

5 Nerve Extension Elicited in Chick Dorsal Root Ganglia by Cyclophilin Ligands

In the present study we have employed explants of chick dorsal root ganglia in order to demonstrate the powerful neurotrophic effects of the cyclophilin binding compounds. The maximal increase in the number
10 of processes, their length and branching is quite similar at maximally effective concentrations of the cyclophilin ligands and of NGF (100 ng/ml). With progressively increasing concentrations of the various drugs, one observes a larger number of processes, more
15 extensive branching and a greater length of individual processes.

We evaluated the potencies of drugs in binding to cyclophilin A by examining inhibition of peptidyl prolyl-isomerase activity (Table 1). There is a
20 striking parallel between their potencies in stimulating neurite outgrowth and inhibiting rotamase activity.

The very close correlation between the potencies of drugs in binding to immunophilins, inhibiting their
25 rotamase activity and stimulating neurite outgrowth implies that inhibition of rotamase activity is responsible for neurotrophic effects of the drugs. The extraordinarily high potency of the drugs in

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stimulating neurite outgrowth and in binding to cyclophilin makes it most unlikely that any other target could account for the neurotrophic effects.

5 Because of the extraordinary potency of the drugs and the close correlation between rotamase inhibition and neurotrophic actions, we conclude that rotamase inhibition of cyclophilin is likely involved in neurotrophic effects.

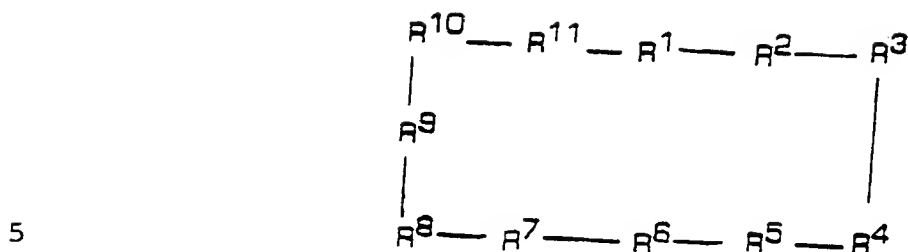
10 The neurotrophic actions of the drugs studied here are exerted at extremely low concentrations indicating potencies comparable to those of neurotrophic proteins such as brain derived growth factor, neurotrophin-3 and neurotrophic growth factor.

15 The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation
20 prepared unless otherwise indicated and all totals equal 100% by weight.

Illustrative cyclophilin inhibitor compounds which can be used for the purposes of this invention include, but are not limited to, the following.

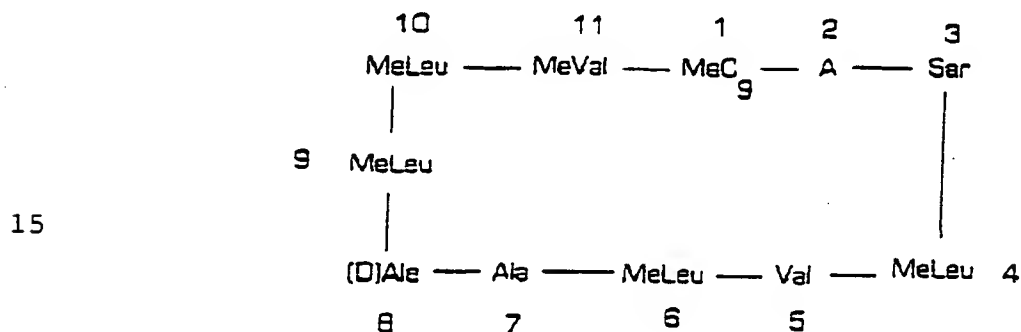
25 Cycloproins of general structural Formula I, comprising cyclic peptides which contain 11 amino acids:

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Formula I

More Particularly, naturally occurring cyclosporins have the general structure shown in
 10 Formula II and embodied in the present invention:

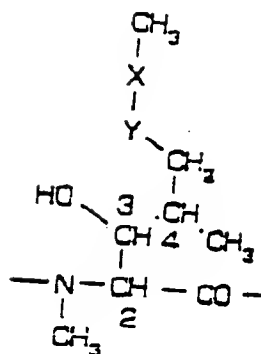


Formula II

wherein "MeC₉" represents the so-called "C9-amino
 20 acid" residue of Formula III (also known as "MeBmt"
 = (4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine):

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5



10

Formula III

in which positions 2, 3, and 4 have the configurations S, R and R, respectively, -X-Y- is -CH=CH- (trans;

15

where

Me = methyl

Abu = a-aminobutyric acid

Val = valine

norVal = norvaline

20

Ala = alanine

MeLeu = N-methyl-leucine

MeVal = N-methyl-valine

Sar = sarcosine

[3-DesoxyMeBmt] = MeBmt wherein the oxygen at position 3 is removed.

25

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Unless specified, the amino acid configuration is L;

and A is,

for cyclosporin A, -Abu-;

5 for cyclosporin B, -Ala-;

for cyclosporin C, -Thr-;

for cyclosporin D, -Val-; and

for cyclosporin G, -norVal-,

all of which are incorporated herein by reference.

10 Additional known naturally occurring cyclosporins are related to cyclosporin A, above, by replacement of an amino acid; thus

for cyclosporin E, position 11 is Val;

for cyclosporin F, position 1 is [3-

15 DesoxyMeBmt]; and

for cyclosporin H, position 11 is [D-MeVal],

all of which are incorporated herein by reference.

In the following discussion, all references to numbered positions in the cyclosporin type structure are with reference to formula II. As is common in the field, a specific cyclosporin analog may be named using the above numbering system, using a shorthand notation to identify how the cyclosporin analog differs from cyclosporin A (CsA). Thus,

20

25 [MeVal]⁴-CsA denotes the cyclosporin in which MeLeu in cyclosporin A (Formula II) has been replaced by MeVal, and so forth.

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In the case of the dihydrocyclosporins, -X-Y- of formula III is $-\text{CH}_2-\text{CH}_2-$. Thus, dihydrocyclosporin A, dihydrocyclosporin C and all other dihydro analogues of naturally occurring as well as semi-synthetic and synthetic cyclosporins are to be understood as falling within the spirit and scope of the invention.

In addition to these known cyclosporins and their dihydro derivatives, a number of non-natural cyclosporins have been prepared by synthetic means, either by total synthesis or synthetic modification of naturally occurring materials, or by modified culture techniques. These synthetic and semi-synthetic cyclosporin analogues and derivatives have been shown to potently inhibit the rotamase activity of cyclophilin, and are thus to be understood as specifically falling within the scope and spirit of the present invention. Such modified cyclosporins include, but are not in any way limited to, the following:

cyclosporins with modified "C9 amino acids"; such modified cyclosporins are disclosed by Witzel (U.S. Patent 4,885,276 and U.S. Patent 4,798,823), and are incorporated herein by reference;

cyclosporins with modified residues at position 8, such as [dehydro-Ala]8-CsA and cyclosporins containing a sulfur-containing amino acid at position-8, as disclosed by Patchett et al. (U.S.

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Patents 5,122,511 and 5,214,130) and incorporated herein by reference;

5 cyclosporins wherein the amino acid at the 8-position is a (D)-acyloxy- α -amino acid residue, as disclosed by Wenger (U.S. Patent 4,764,503) and incorporated herein by reference;

10 cyclosporins having an α -hydroxycarboxylic acid at position-8, as described by Dreyfuss et al. (U.S. Patent 5,116,816) and incorporated herein by reference;

15 cyclosporins containing hydroxy-substituted serine residue at position-8, such as [O-(2-hydroxyethyl) (D)Ser]⁸-CsA, are disclosed by Eberle in U.S. Patent 5,284,826 and incorporated herein by reference;

 cyclosporins containing one or more fluorinated amino acids, as for example described by Durette et al. in U.S. Patent 5,227,467 and incorporated herein by reference;

20 cyclosporins which contain at the 3-position an optically active, N-methylated α -amino acid residue of the (D) configuration, as disclosed by Seebach in U.S. Patent 4,703,033 and incorporated herein by reference;

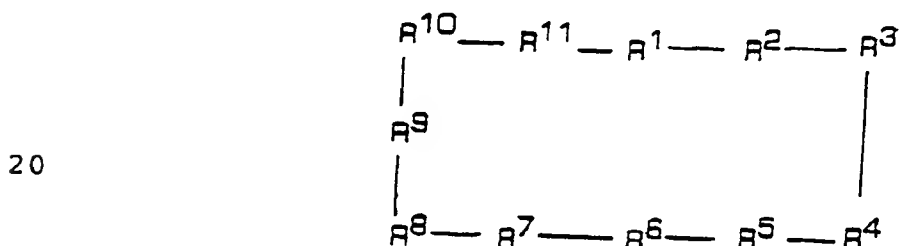
25 cyclosporins which contain an allyl-Gly residue in the 2-position and/or a -(D)-Ser residue in the 8-position, as disclosed by Bollinger et al. (U.S.

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Patent 4,384,996) and incorporated herein by reference.

In addition to the above cyclosporins and cyclosporin analogs which possess cyclophilin inhibitory and immunosuppressive activity, it is also an intention of the present invention to provide a method for the use of non-immunosuppressive cyclosporin analogs as neurotrophic agents. Such non-immunosuppressive cyclosporin analogs bind to cyclophilin and inhibit its rotamase activity, while not eliciting immunosuppression, and are potent neurotrophic agents.

The following structural Formula IV is illustrative of such embodiment, and is not meant to be construed as limiting the scope of the invention in any way:



Formula IV

wherein

- 25 R^1 is MeBmt or dihydro MeBmt;
 R^2 is Abu or a fluorinated analog thereof;

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R³ is Sar, D-MeAla, or a fluorinated analog thereof;

5 R⁴ is an N-methylated amino acid residue with a (C1-C9) straight or branched chain alkyl or alkenyl group; these straight or branched alkyl or alkenyl groups may be substituted by cycloalkyl (C3-C8); R₁ may also be (C3-C8) cycloalkyl or (C5-C7) cycloalkenyl; the above
10 alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be substituted with (C1-C4) alkyl or (C1-C4) alkenyl, or hydroxy;

R⁹ and R¹⁰ are independently MeLeu or a fluorinated analog thereof;

R⁵ is Val or a fluorinated analog thereof;

15 R⁶ is MeAla, MeAbu, or a fluorinated analog thereof;

R⁷ is Ala or a fluorinated derivative thereof;

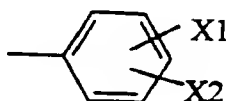
R⁸ is

(a) D-Ala or a fluorinated analog thereof;

20 or

(b) O-acyl-D-Ser or O-acyl-D-Thr wherein the acyl group is defined as R¹² -CO- where R¹² represents hydrogen, C₁₋₆alkyl, phenyl or substituted phenyl of formula V:

25



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wherein X^1 and X^2 independently are

- (a) C_{1-6} alkyl;
- (b) C_{1-6} alkanoyl;
- (c) CH_2OH ;
- 5 (d) halo;
- (e) C_{1-6} alkoxy;
- (f) $-NH_2$;
- (g) $-NO_2$;
- (h) $-COOH$;
- 10 (i) $-COOC_{1-6}$ alkyl; or
- (j) $-H$;

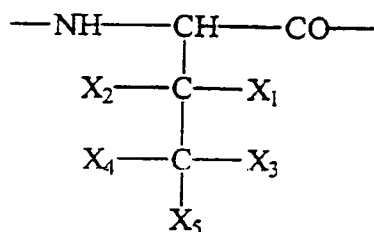
Particularly preferred are cyclosporin analogs
wherein

- 15 R^1 is MeBmt or dihydro MeBmt;
- R^2 is Abu;
- R^3 is Sar or D-MeAla;
- R^4 is MeLeu, MeVal, Me homo-Ala, or [Me-(α -methyl)Thr];
- 20 R^9 and R^{10} are independently MeLeu or a fluorinated analog thereof;
- R^5 is Val or a fluorinated analog thereof;
- R^6 is MeAla, MeAbu, or a fluorinated analog thereof;
- 25 R^7 is Ala;
- R^8 is D-Ala; and
- R^{11} is MeVal or a fluorinated analog thereof.

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Fluorinated analogs represent the amino acid residues wherein one or more of the various C-H bonds in the side chain are replaced with C-F bonds. For example, fluorinated analog of Abu represents

5



10 wherein X_1 to X_5 independently are H or F with the proviso that at least one of X_1 , X_2 , X_3 , X_4 , and X_5 is F.

Known derivatives and analogs of cyclosporins, which are known to be potent inhibitors of immunophilin rotamase activity and lack immunosuppressant effects include modified 6-position analogs, described by Dumont et al. (U.S. Patent 4,914,188) and Durette (U.S. Patent 5,236,899) and incorporated herein by reference.

15 Other known non-immunosuppressive cyclosporin analogs include N-alkylated [Val]⁴ derivatives, such as [MeVal]⁴-CsA, disclosed by Fliri et al. (Annal. N.Y. Acad. Sci 696, 47, 1993), and incorporated herein by reference, and other derivatives with various side chain on the 4-position residue, as described by Papageorgiou et al. (BioMed. Chem. Lett. 1994, 2, 267-272) and incorporated herein by reference.

20

25

K_i Test Procedure

Inhibition of the peptidyl-prolyl isomerase (rotamase) activity of cyclophilin by the inventive compounds can be evaluated by known methods described in the literature (Harrison and Stein, Biochemistry, 1990, 29, 3813-3816). These values are obtained as apparent K_i's and are presented for some of Examples 1-30 in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as a function of inhibitor concentration to yield the apparent K_i values. The absorbance at 390 nm versus time is monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

The data for these experiments is presented in Table I.

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TABLE I

	Example inhibition	Cyclosporin	Ki, cyclophilin
5	1	CsA	20 nM
	2	dihydro-CsA	100 nM
	3	[dehydro-Ala] ⁸ -CsA	75 nM
10	4	[MeVal] ⁴ -CsA	10 nM
	5	[Me-homoAla] ⁴ -CsA	24 nM
	6	[Me-(α -methyl)Thr] ⁴ -CsA	18 nM

15

Chick Dorsal Root Ganglion
Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μ M cytosine β -D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO₂. Twenty-four hours later, the DRGs were treated with various concentrations of cyclosporin drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate.

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The data for these experiments are presented in Table II. Referring to FIG. 1 of the drawings, the ED₅₀ for cyclosporin A, the dose at which 50% of the maximal response was elicited, was obtained from dose-response curves (Fig. 1 is a typical example) and calculated to be 50 nM. The relative potencies of the other compounds in Table II are given relative to cyclosporin A, the number of "+" marks denoting the relative potency.

Table II

Neurite Outgrowth in Chick DRG

Example	Neurotrophic Potency
1	+++
2	+
3	+
4	+++
5	+++
6	+++

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The above data demonstrate that compounds which are inhibitors of cyclophilin's rotamase activity, whether immunosuppressive or non-immunosuppressive, are capable of promoting neurite outgrowth in
5 cultured neurons, and are capable of achieving maximal effects comparable to nerve growth factor itself.

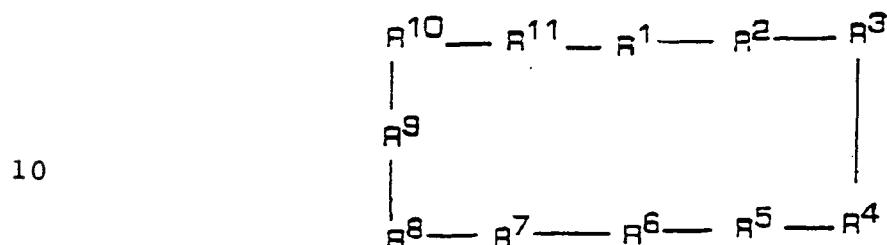
The invention being thus described, it will be obvious that the same may be varied in many ways.
10 Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

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WHAT IS CLAIMED IS:

1. A method of treating a neurological activity in an animal, comprising:

administering to an animal an effective amount of an immunosuppressive cyclosporin represented by formula I:

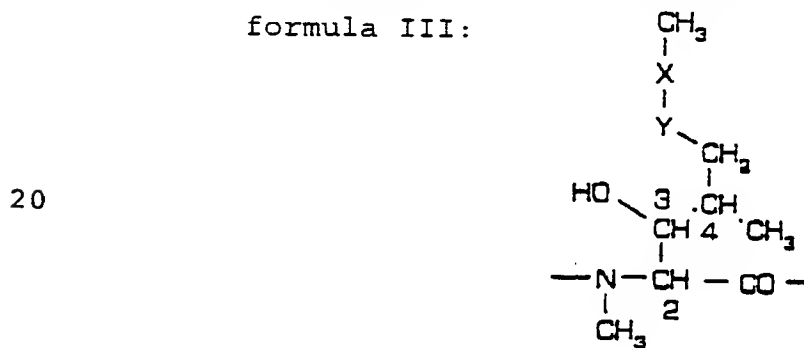


I

and pharmaceutically acceptable salts thereof

15 wherein

R^1 is MeBmt or [3-DesoxyMeBmt] or represented by formula III:



III

25 wherein positions 2, 3, and 4 have the configurations S, R and R, respectively;

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wherein -X-Y- is connected by a double bond or a single bond;

wherein:

5 R² is independently selected from the group consisting of Abu, Ala, Thr, Val, and norVal;

R³ is Sar;

R⁴ is MeLeu;

R⁵ is Val;

R⁶ is MeLeu;

10 R⁷ is Ala;

R⁸ is D-Ala;

R⁹ is MeLeu;

R¹⁰ is MeLeu; and,

R¹¹ is Val or [D-MeVal].

- 15 2. The method of claim 1 wherein R¹ is [3-DesoxyMeBmt] for cyclosporin F.
3. The method of claim 1 wherein R² is Abu for cyclosporin A.
4. The method of claim 1 wherein R² is Ala for
20 cyclosporin B.
5. The method of claim 1 wherein R² is Thr for cyclosporin C.
6. The method of claim 1 wherein R² is Val for cyclosporin D.
- 25 7. The method of claim 1 wherein R² is norVal for cyclosporin G.
8. The method of claim 1 wherein R¹¹ is Val for cyclosporin E.

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9. The method of claim 1 wherein R¹¹ is [D-MeVal] for cyclosporin H.
10. The method of claim 1 wherein cyclosporin has a modified "C9 amino acid" at position R¹.
- 5 11. The method of claim 10 wherein the modified "C9 amino acid" at position R¹ is fluorinated MeBmt.
12. The method of claim 1 wherein R² is an allyl-Gly residue.
13. The method of claim 1 wherein R² is an allyl-Gly residue and wherein R⁸ is a (D)-Ser residue.
- 10 14. The method of claim 1 wherein R³ is an optically active, N-methylated α -amino acid residue of the (D) configuration.
- 15 15. The method of claim 1 wherein cyclosporin has a modified amino acid at position R⁸.
16. The method of claim 15 wherein R⁸ is dehydro-Ala or O-(2-hydroxyethyl) (D) Ser.
17. The method of claim 15 wherein R⁸ is a (D)-Ser residue.
- 20 18. The method of claim 15 wherein R⁸ is a modified sulfur-containing amino acid.
19. The method of claim 15 wherein R⁸ is a (D)-acyloxy- α -amino acid residue.
20. The method of claim 15 wherein R⁸ is an α -hydroxycarboxylic acid.
- 25 21. The method of claim 15 wherein R⁸ is a hydroxy-substituted serine residue.

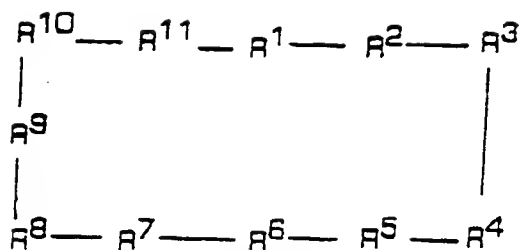
-36-

22. The method of claim 1 wherein -X-Y- of formula II is a double bond in a trans configuration.
23. The method of claim 1, wherein the neurological activity is stimulation of damaged neurons.
- 5 24. The method of claim 1, wherein the neurological activity is stimulation of growth of damaged peripheral nerves.
25. The method of claim 1, wherein the neurological activity is stimulation of growth of damaged neurons in the spinal cord.
- 10 26. The method of claim 1, wherein the neurological activity is stimulation of growth of motor neurons.
27. The method of claim 1, wherein the neurological activity is promotion of neuronal degeneration.
- 15 28. The method of claim 1, wherein the neurological activity is prevention of neurodegeneration.
29. The method of claim 1, wherein the neurological activity is treatment of neurological disorders.
- 20 30. The method of claim 29, wherein the neurological disorder is peripheral neuropathies caused by physical injury or disease state.
- 25 31. The method of claim 29, wherein the neurological disorder is physical damage to the brain.

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32. The method of claim 29, wherein the neurological disorder is physical damage to the spinal cord.
- 5 33. The method of claim 29, wherein the neurological disorder is stroke associated with brain damage.
34. The method of claim 29, wherein the neurological disorder relates to neurodegeneration.
- 10 35. The method of claim 29, wherein the neurological disorder is Alzheimer's Disease.
36. The method of claim 29, wherein the neurological disorder is Parkinson's Disease.
- 15 37. The method of claim 1 further including a neurotrophic factor in amounts sufficient to treat the neurological activity.
38. The method of claim 37 wherein the neurotrophic factor is selected from the group consisting of nerve growth factor, glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotrophin-3.
- 20 39. A method of treating a neurological activity in an animal, comprising:
administering to an animal an effective amount
25 of a non-immunosuppressive cyclosporin of formula IV:

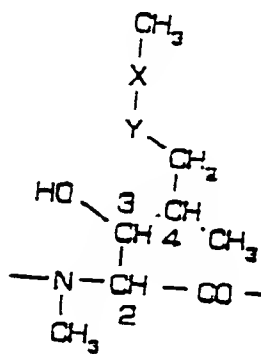
-38-



Formula IV

wherein:

R^1 is MeBmt or dihydro MeBmt or represented by formula III:



Formula III

wherein positions 2, 3, and 4 have the configurations S, R and R, respectively;

wherein -X-Y- is connected by a double bond or a single bond;

wherein:

R^2 is Abu or a fluorinated analog thereof;

R^3 is Sar, D-MeAla, or a fluorinated analog thereof;

R^4 is an N-methylated amino acid residue with a (C1-C9) straight or branched chain alkyl or alkenyl group; these straight or branched alkyl or alkenyl groups may be substituted by cycloalkyl (C3-C8); R_1 may also be (C3-C8)

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cycloalkyl or (C5-C7) cycloalkenyl; the above alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be substituted with (C1-C4) alkyl or (C1-C4) alkenyl, or hydroxy;

5 R^5 is Val or a fluorinated analog thereof;

R^6 is MeAla, MeAbu, or a fluorinated analog thereof;

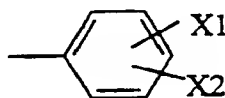
R^7 is Ala or a fluorinated derivative thereof;

R^8 is

10 (a) D-Ala or a fluorinated analog thereof;

or

(b) O-acyl-D-Ser or O-acyl-D-Thr wherein the acyl group is defined as R^{12} -CO- where R^{12} represents hydrogen, C_{1-6} alkyl, phenyl
15 or substituted phenyl of formula V:



20

Formula V

wherein X^1 and X^2 independently are

(a) C_{1-6} alkyl;

(b) C_{1-6} alkanoyl;

25 (c) CH_2OH ;

(d) halo;

(e) C_{1-6} alkoxy;

(f) $-NH_2$;

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(g) $-\text{NO}_2$;(h) $-\text{COOH}$;(i) $-\text{COOC}_{1-6}\text{alkyl}$; or(j) $-\text{H}$;

- 5 R^9 and R^{10} are independently MeLeu or a fluorinated analog thereof; and,
 R^{11} is MeVal or a fluorinated analog thereof.
40. The method of claim 39 wherein R^4 is MeLeu, MeVal, Me homo-Ala, or [Me-(α -methyl)Thr].
- 10 41. The method of claim 39, wherein the neurological activity is stimulation of damaged neurons.
42. The method of claim 39, wherein the neurological activity is stimulation of growth
15 of damaged peripheral nerves.
43. The method of claim 39, wherein the neurological activity is stimulation of growth
 of damaged neurons in the spinal cord.
44. The method of claim 39, wherein the
20 neurological activity is stimulation of growth of motor neurons.
45. The method of claim 39, wherein the neurological activity is promotion of neuronal degeneration.
- 25 46. The method of claim 39, wherein the neurological activity is prevention of neurodegeneration.

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47. The method of claim 39, wherein the neurological activity is treatment of neurological disorders.
- 5 48. The method of claim 39, wherein the neurological disorder is peripheral neuropathies caused by physical injury or disease state.
- 10 49. The method of claim 39, wherein the neurological disorder is physical damage to the brain.
50. The method of claim 39, wherein the neurological disorder is physical damage to the spinal cord.
- 15 51. The method of claim 39, wherein the neurological disorder is stroke associated with brain damage.
52. The method of claim 39, wherein the neurological disorder relates to neurodegeneration.
- 20 53. The method of claim 39, wherein the neurological disorder is Alzheimer's Disease.
54. The method of claim 39, wherein the neurological disorder is Parkinson's Disease.
- 25 55. The method of claim 39 further including a neurotrophic factor in amounts sufficient to treat the neurological activity.
56. The method of claim 55 wherein the neurotrophic factor is selected from the group consisting of

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nerve growth factor, glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotrophin-3.

57. The method of claim 39, wherein the cyclosporin
5 is Cyclosporin A.
58. The method of claim 58 wherein R⁴ is selected from the group consisting of MeVal, Me[homo-Ala], and Me-(α -methyl)Thr.

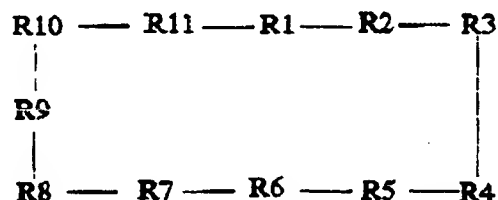
AMENDED CLAIMS

[received by the International Bureau on 9 May 1997 (09.05.97);
original claims 1-58 replaced by amended claims 1-20 (4 pages)]

1. A method of treating a neurological activity in an
animal, comprising:

administering to an animal an effective amount of a

5 non-immunosuppressive cyclosporin of formula IV:

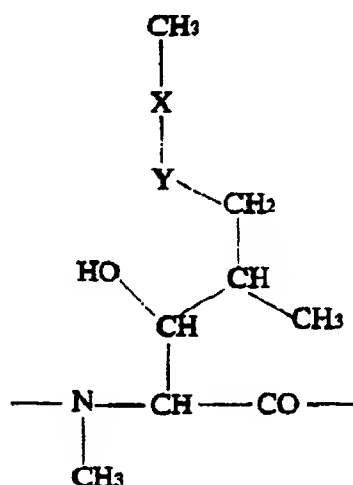


Formula IV

wherein:

R^1 is MeBmt or dihydro MeBmt or represented by

10 formula III:



Formula III

wherein positions 2, 3, and 4 have the
15 configurations S, R and R, respectively;

wherein -X-Y- is connected by a double bond or a
single bond;

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wherein:

R² is Abu or a fluorinated analog thereof;

R³ is Sar, D-MeAla, or a fluorinated analog thereof;

R⁴ is an N-methylated amino acid residue with a (C1-

5 C9) straight or branched chain alkyl or alkenyl group; these straight or branched alkyl or alkenyl groups may be substituted by cycloalkyl (C3-C8); R₁ may also be (C3-C8) cycloalkyl or (C5-C7)

10 cycloalkenyl; the above alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be substituted with (C1-C4) alkyl or (C1-C4) alkenyl, or hydroxy;

R⁵ is Val or a fluorinated analog thereof;

R⁶ is MeAla, MeAbu, or a fluorinated analog thereof;

R⁷ is Ala or a fluorinated derivative thereof;

15 R⁸ is

(a) D-Ala or a fluorinated analog thereof; or

(b) O-acyl-D-Ser or O-acyl-D-Thr wherein the

acyl group is defined as R¹² -CO- where R¹²

represents hydrogen, C₁₋₆alkyl, phenyl or

20 substituted phenyl of formula V:



Formula V

wherein X¹ and X² independently are

(a) C₁₋₆alkyl;

25 (b) C₁₋₆alkanoyl;

(c) CH₂OH;

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- (d) halo;
- (e) C₁₋₆alkoxy;
- (f) -NH₂;
- (g) -NO₂;
- 5 (h) -COOH;
- (i) -COOC₁₋₆alkyl; or
- (j) -H;

R⁹ and R¹⁰ are independently MeLeu or a fluorinated analog thereof; and,

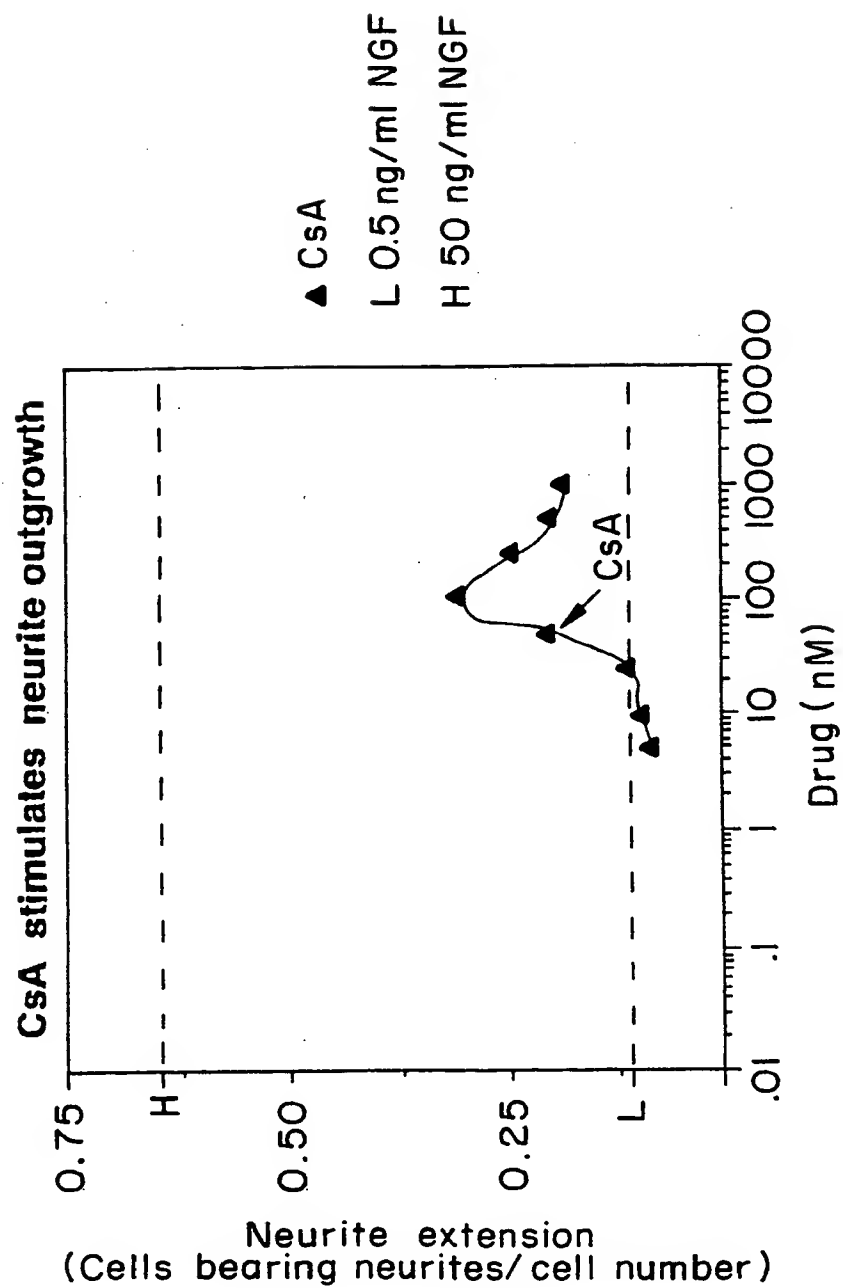
- 10 R¹¹ is MeVal or a fluorinated analog thereof.
- 2. The method of claim 1 wherein R⁴ is MeLeu, MeVal, Me homo-Ala, or [Me-(α -methyl)Thr].
- 3. The method of claim 1, wherein the neurological activity is stimulation of damaged neurons.
- 15 4. The method of claim 1, wherein the neurological activity is stimulation of growth of damaged peripheral nerves.
- 5. The method of claim 1, wherein the neurological activity is stimulation of growth of damaged neurons
- 20 in the spinal cord.
- 6. The method of claim 1, wherein the neurological activity is stimulation of growth of motor neurons.
- 7. The method of claim 1, wherein the neurological activity is promotion of neuronal degeneration.
- 25 8. The method of claim 1, wherein the neurological activity is prevention of neurodegeneration.
- 9. The method of claim 1, wherein the neurological activity is treatment of neurological disorders.

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10. The method of claim 1, wherein the neurological disorder is peripheral neuropathies caused by physical injury or disease state.
11. The method of claim 1, wherein the neurological disorder is physical damage to the brain.
12. The method of claim 1, wherein the neurological disorder is physical damage to the spinal cord.
13. The method of claim 1, wherein the neurological disorder is stroke associated with brain damage.
- 10 14. The method of claim 1, wherein the neurological disorder relates to neurodegeneration.
15. The method of claim 1, wherein the neurological disorder is Alzheimer's Disease.
16. The method of claim 1, wherein the neurological disorder is Parkinson's Disease.
- 15 17. The method of claim 1 further including a neurotrophic factor in amounts sufficient to treat the neurological activity.
18. The method of claim 17 wherein the neurotrophic factor is selected from the group consisting of nerve growth factor, glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotrophin-3.
- 20 19. The method of claim 1, wherein the cyclosporin is Cyclosporin A.
- 25 20. The method of claim 19 wherein R' is selected from the group consisting of MeVal, Me[homo-Ala], and Me-(α -methyl)Thr.

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FIG. 1



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US96/17677
A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/13, 38/00; C12P 21/00

US CL : 530/333; 514/11; 435/71.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/333, 317, 321, 334, 335; 514/11; 435/71.1, 254.1; 930/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
THE MERCK INDEXElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, WPIDS, CAPLUS, REGISTRY, EMBASE**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	SNYDER et al. Nature Medicine. "Immunophilins and the nervous system", January 1995, Vol. 1. No. 1. pages 32-37.	1-2, 4, 7-9, 11, 22-38, 41-56 ----- 3, 5-6, 10, 12-21, 39-40, 57
Y	US 4,885,276 A (WITZEL) 05 December 1989, see the entire document.	3, 5, 6
Y	US 4,764,503 A (WENGER) 16 August 1988, see the entire document.	10, 15-17, 22
Y	US 4,384,996 A (BOLLINGER et al.) 24 May 1983, see the entire document.	12-13

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

 Date of the actual completion of the international search
 05 FEBRUARY 1997

 Date of mailing of the international search report
 11 MAR 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17677

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,703,033 A (SEEBACH) 27 October 1987, see the entire document.	14
Y	US 5,122,511 A (PATCHETT et al.) 16 June 1992, see the entire document.	18
Y	US 5,116,816 A (DREYFUSS et al.) 26 May 1992, see the entire document.	20
Y	US 4,914,188 A (DUMONT et al.) 03 April 1990, see the entire document.	39-40, 57
Y	US 5,284,826 A (EBERLE) 08 February 1994, see the entire document.	21